Up to date more than 70 different viruses are found in grapevine, making it a good model plant for investigation of viral infection. The aim of this study was to compare accuracy and reliability of different NGS-based methods in their detection.

**Materials and Methods**

In this study two grapevine accessions were used: VB-108 (variety Babička) and VLJ-178 (var. Lutjan). Total RNA was isolated in August 2015 from 0.1 g of leaf petioles using a RNeasy Plant Mini Kit (Qiagen, Germany). High ribosomal RNA (rRNA) was depleted with the Illumina Ribo-Zero rRNA Removal Kit - Plant (Illumina, USA). Sequencing libraries were constructed at Functional Genomics Lab at QB3-Berkeley Core Research Facility (UC Berkeley) on an Apollo 324™ with PrepX™ RNAseq Library Prep Kits (WaferGen Biosystems, USA). Thirteen cycles of PCR amplification were used for index addition and library fragment enrichment. Genomic data, 100 paired-end reads, were generated on a HiSeq4000 platform (Illumina, USA) at Vincent J. Coates Genomics Sequencing Laboratory (UC Berkeley). The FastQ program (Babraham Bioinformatics, UK) was used for initial data quality control, and reads were trimmed and filtered with Sickle using default parameters. Trimmed reads were aligned to a grapevine genome (www.genoscope.cns.fr) using Bowtie 2 ver. 2.2.9 and unaligned (host genome filtered) reads were extracted, sorted using SAMtools ver. 1.3.1, and converted to FASTQ file format using BEDTools ver. 2.26.0 for analysis.

Unaligned reads were used for de novo assembly with SPAdes ver. 3.9.0; parameters used included k-mer values 21, 33, 55 and 75, and the careful option for reduction of mismatches and short indels. All assembled contigs were subjected to a remote BLAST nucleotide search for viruses with cut-off e-value set at e-20 (query terms: "viruses NOT plants"). In addition to the de novo assembly, trimmed reads were subject to: i) mapping to a custom-made library of grapevine-associated viruses using Bowtie 2; ii) search by automated e-probe-based software Truffle and iii) VirFind with default e-value (0.01) and conserved domains search option.

To validate some of the NGS-based results for recently reported grapevine viruses (GVB-1, GRLDaV, GVG, GVK and GVt), plants were resampled in September 2017 using RNeasy/DEasy Plant Mini Kit. Based on sequences generated by NGS specific primers were designed and their RT-PCR or PCR products were verified by Sanger sequencing at Macrogen (Korea).

**Results and Discussion**

All NGS-based methods compared in this study were effective in detecting most viruses present in samples. Discrepancies in NGS- and PCR-results done prior to NGS could be due to primers/probes design based on already known sequences of certain pathogens that could be less effective in detection of genetically different and not yet described virus variants. This was also confirmed by PCR-based cross-validations tests done for GVB-1, GVG, GVK, GVT and GRLDaV using primers designed from the NGS-data obtained in survey. Although read-mapping showed to be relatively fast and accurate, using adequate cut-off thresholds is challenging. Sometimes large number of reads mapped could lead to problematic ‘positive’ or false positive results (ArMV, GBLV, GDeFv, GGLRaV, GVt/VaV), which indicated false negative results. In such cases, including more virus variants to custom-made library could provide more accurate results. VirFind showed to be a well-balanced tool for detection of known viruses, but in general generated shorter contigs when compared to de novo assembly using SPAdes. Truffle proved to be an efficient and fast tool for detection of known viruses with limitations in the detection of viruses with limited sequence information (e.g. GVD).

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